Effect of controlled atmosphere on the activity and kinetics of three detoxification enzymes in *Araecerus fasciculatus* (Coleoptera: Anthribidae)

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Abstract: To clarify the effect of controlled atmosphere (CA) on stored pests and enzyme activities related to the development of resistance, the effect of CA with enriched CO_2 (75% CO_2, 5% O_2 and 20% N_2) on the activities of carboxylesterase (CarE), acid phosphatase (ACP) and glutathione S-transferases (GSTs) from an experimental population of *Araecerus fasciculatus* (De Geer) were investigated. The results showed that CarE activities were significantly affected by CA with exposure time, increased by 35.41%, 55.02% and 88.98% at 3 h, 6 h and 9 h, respectively, after exposure compared with the control. The CA treatment increased the Michaelis-Menten (M-M) kinetic constant, V_{max}, of CarE by 26.13%, 31.77% and 57.12%, respectively at 3 h, 6 h and 9 h after exposure, but had no effect on the M-M constant, K_m. CA treatments of 3 h, 6 h and 9 h increased the specific activity of ACP by 34.53%, 72.45% and 126.37%, respectively. Compared to the control, the specific activities of GSTs in adults exposed to CA for 3 h, 6 h and 9 h were increased by just 5.40%, 8.40% and 17.59%, respectively. These results suggest that *A. fasciculatus* can adjust the activity of some detoxification enzymes and the kinetics of CarE-catalyzed reactions under the stress of CA. Under CA conditions, the catalytic activity of some detoxification enzymes towards their substrates were enhanced, potentially providing the ability for the pest to adjust its metabolic activity against extreme conditions. This study provides insights into insect responses to CA treatment and the mechanism of CA resistance or tolerance.

Key words: Stored product pest; *Araecerus fasciculatus*; controlled atmosphere (CA); resistance to controlled atmosphere; detoxification enzyme; enzyme activity; enzyme kinetics

1 INTRODUCTION

Controlled atmosphere (CA) with low O_2 and elevated CO_2 is insecticidal and has been used commercially to control stored product insect pests in dry commodities. Studies have shown that insects exposed to CA for successive generations may develop resistance in a similar manner to insecticides and fumigants (Bond and Buckland, 1979; Navarro *et al.*, 1985; Wang *et al.*, 1999, 2000). Understanding the mechanism of CA resistance is essential to the sustainability of this control tactic. Knowledge of the mode of action often helps to delineate the mechanism of tolerance or resistance. Studies have indicated that the insecticidal CA mode of action is complex, involving desiccation, respiration, energy metabolism, membrane permeability and hypoxia (Ofuya and Reichmuth, 1998; Cheng *et al.*, 2001; Zhang *et al.*, 2003; Cheng *et al.*, 2005). The reduction in metabolism lessens the pressure on the organism to initiate anaerobic metabolism, also leads to a reduction in ATP production (Mitcham *et al.*, 2006). Reduced O_2 consumption leads to a decreased rate of ATP production. The cell and mitochondrial membranes become more permeable, leading to cell damage or death (Hochachka, 1986). Minutes after treatment with anoxia, ATP levels declined and ADP, AMP, and IMP (inosine monophosphate) levels increased (Hoback and Stanley, 2001). *Platynota stultana* Walsingham (Lepidoptera: Tortricidae) pupae used

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metabolic arrest as a major response to hypoxia (Zhou *et al.*, 2001). However, the mechanisms underlying survival in such extreme conditions are not currently clear (Zhou *et al.*, 2008). Under elevated CO_2 and/or low O_2 conditions, the lower rate of metabolism and initiation of anaerobic respiration are stressful to insects (Hochachka, 1986). The survival of insects under a CA treatment is likely to depend on their tolerance and/or the ability to detoxify toxic metabolic end products (Kennington and Cannel, 1967), implying the importance of detoxification enzymes.

Adaptations to hypoxia include the ability to switch from aerobic to anaerobic metabolic pathways, the ability to drastically attenuate basal metabolic rates, altered behaviors and enlarged tracheal system volumes (Hoback and Stanley, 2001, Yong *et al.*, 2011). This is a different responsive mechanism from enzyme system function of pest stressed pesticides (Margam, 2009). Carboxylesterase (CarE), acid phosphatase (ACP) and glutathione S-transferases (GSTs) are three detoxification enzymes in insects. Some references suggested that the activity of some enzymes in insects could be affected by CA treatments. Improved activity and changed kinetics index of several detoxification enzymes in stored pests may result from extended exposure to CA, such as CarE (Li *et al.*, 2009b), AChE (Li *et al.*, 2007), ACP (Li *et al.*, 2008), GSTs (Li and Li, 2009) and so on. The resistance to CAs and insecticides is correlated to some extent (Ding *et al.*, 2004),

The coffee bean weevil, *Araecerus fasciculatus* (De Geer), is a widely distributed insect pest that attacks a wide variety of agricultural products both in the field and in storage, including coffee beans, corn, various seeds, stored dry roots, spices and dried fruits (Soans and Soans, 1972). In China, this weevil is a serious pest in storage facilities for traditional Chinese medicine materials (CMM), which are mostly dried herbs (Li *et al.*, 2009a). Due to the intended use of CMM (for human consumption), CA is an appealing means of insect control for storage facilities, because CA is a safe and residual-free alternative. In this study, using *A. fasciculatus* as a model organism, we investigated the effect of a CA ($75\% \pm 5\%$ CO_2 and $25\% \pm 5\%$ natural atmosphere) on the specific activities of CarE, ACP nand GSTs, and studied the effect on the kinetic parameters of CarE. The results provide insights into insect responses to CA treatment and the mechanism of CA resistance.

2 MATERIAL AND METHODS

2.1 Insects

A laboratory colony of *A. fasciculatus* was established from 200 larvae collected at a wholesale CMM market in Guiyang, Guizhou Province, China, in April, 2007. The insects were reared for 8 generations with dried Chinese date (*Ziziphus zizyphus*), packaged in sacks in an air-controlled insect rearing chamber at $29℃ \pm 1℃$, $75\% \pm 5\%$ relative humidity (RH) and photoperiod of 14L: 10D before the test.

2.2 Controlled atmosphere and bioassay

In order to determine the effect of CA on several detoxification enzyme properties, a controlled atmosphere with 75% CO_2, 5% O_2 and 20% N_2 was created by a controlled atmosphere generator (Mix, Changjin S&T Ltd., Changsha, China) (Li *et al.*, 2008, 2009b). The efficacy of CA against the beetle was determined using a small airtight glass box (20 cm × 30 cm × 20 cm). Various exposure periods to the CA were tested until a satisfactory mortality range (5% −99%) was achieved (Li *et al.*, 2009b). Six exposure periods were used in the final analysis.

Each bioassay consisted of 60 adults and six exposure periods (3 h, 6 h, 9 h, 12 h, 15 h and 18 h). Control groups were exposed to normal atmospheric conditions. Mortality was assessed after insects were transferred to natural atmospheric conditions for 6 h. Insects that did not move after being probed with a camel's hair brush were scored as dead (Li *et al.*, 2009b). All tests were performed at 29℃ and replicated at least five times on different days. Mortality data was corrected with Abbott (1925) formula and analyzed by probit analysis (Raymond, 1985) to determine the median mortality time (LT_{50}).

The effect of the CA on activities of three detoxification enzymes and their kinetic parameters were evaluated by using extracts prepared from *A. fasciculatus* adults subjected to CA treatment for 0 h, 3 h, 6 h and 9 h, respectively. Enzyme extracts were prepared as described below and enzyme activity and the kinetic parameters were measured and compared with those of the control. Three replicates were prepared for the treatment and the control.

2.3 Enzyme extraction

2.3.1 CarE: The procedure described in Cheng *et al.* (2001) was followed for CarE extract preparation. Briefly, 20 adults (3 − 5 d old) were homogenized in a glass homogenizer with 0.4 mL ice-cold sodium phosphate buffer (0.04 mol/L PBS, pH 7.0) on ice, followed by addition of 7.6 mL PBS. The homogenates were divided equally into eight microcentrifuge tubes (1.5 mL) and centrifuged at 10 000 r/min (by centrifuge, 3K30, Sigma®, St.

Louis, MO, USA), at 4℃ for 15 min. The supernatants containing CarE were collected and tested immediately.

2.3.2 ACP: Extracts were prepared by homogenizing thirty adults in 0.4 mL ice-cold acetate buffer (0.2 mol/L, pH 4.6) in an ice water bath. The subsequent steps were the same as for CarE.

2.3.3 GSTs: Thirty 3–5 d old adults were homogenized with 0.4 mL PBS. All other extraction steps and CA treatments were the same as for CarE.

2.4 Enzyme assays

2.4.1 CarE activity and kinetics: Protein contents of enzyme extracts were quantified with a standard curve generated using bovine serum albumin (Roche®) by coomassie brilliant blue (G-250, Sigma®) method (Bradford, 1976). Esterase activity was measured according to the method of van Asperen (1962) using α-NA (Sigma®) as a substrate. Before being tested, enzyme extracts were diluted with PBS to a concentration with optical density (OD) readings between 0.2 and 0.8. The reaction systems contained 5 mL α-NA (3×10^{-4} mol/L, in acetone), 0.03 mL serine (Fluka®) (1×10^{-4} mol/L, in acetone), and 0, 0.1, 0.3, 0.5, 0.7, 0.9 or 1.0 mL of the extracts. The final volume was adjusted to 6.03 mL with PBS. All solutions were incubated in a 37℃ water bath for 30 min. The reaction was terminated with 1 mL of 1% Fast Blue B salt in sodium dodecyl sulfate solution. After 10 min of equilibration under room temperature, the absorbance was read with a microplate reader (Sunrise™, Tecan Group Ltd) at 600 nm. Specific activity was calculated and expressed as mmol/mg · min.

The Michaelis-Menten (M-M) kinetics rate constant, V_{max}, and M-M constant, K_m, of CarE after exposure to CA treatments was determined using the Lineweaver-Burk plot. The total reaction volume was 6.03 mL, containing 0.6 mL of enzyme extracts, 0.03 mL eserine (1×10^{-4} mol/L, in acetone), and α-NA at final concentrations adjusted to 0.025, 0.05, 0.1, 0.2, 0.5 or 1.0 mol/L (Han *et al.*, 1998).

2.4.2 ACP activity: ACP activities were determined from a standard curve generated using p-nitrophenol (Tianjin, China). The reaction systems contained 0.5 mL 4-nitrophenyl phosphate disodium salt (Amresco®) (5×10^{-4} mol/L, in acetone and water) and 0, 0.1, 0.2, 0.3, 0.4, 0.5 or 0.6 mL of extracts. The total reaction volume was adjusted to 3 mL with 0.2 mol/L acetate buffer solution. All solutions were incubated in a 37℃ water bath for 30 min. The reaction was terminated with 2.0 mL of NaOH solution (0.2 mol/L). After 10 min of equilibration under room temperature, the absorbance was read at 400 nm (Sunrise™, Tecan Group Ltd.).

2.4.3 GSTs activity: GSTs activity was measured using 1-chloro-2, 4-dinitrobenzene (CDNB, Sigma®) as a substrate following the method described by Cheng (2006). Briefly, the reaction system of 3 mL consisted of 0.1 mL CDNB (50 mmol/L), 0.1 mL reduced glutathione (GSH, mmol/L) (Amresco®), 2.5 mL enzyme extracts and 0.3 mL PBS (0.1 mol/L, pH 6.5). The control sample consisted of 0.1 mL CDNB (50 mmol/L), 0.1 mL GSH (mmol/L), 2.8 mL PBS (0.1 mol/L, pH 6.5) with no enzyme extract. The systems were scanned at 340 nm for 5 min at 25℃, with three replications. The activity was calculated as follows:

$$\text{GSTs activity} = \Delta OD_{340} \times V / (\varepsilon \times L) \text{ (unit/nmol · min)}$$

where V = reaction volume (3 mL), ε = 9.6 nmol/L · cm (extinction coefficient of the reaction product), and L = 1 cm (size of cuvette).

2.5 Data analysis

Linear regression equations were generated in MS Excel™ (version Microsoft Office™ Excel 2003) for all standard curves and reaction curves to determine enzyme activity (slope). One-way ANOVA was performed to evaluate the effect of the CA treatment on the specific activity and kinetic parameters of CarE, followed by Duncan's multiple range test for mean separation. All statistical procedures were performed using SPSS 18.0.

3 RESULTS

3.1 Bioassays

The LT_{50} value for adults exposed to the CA was 11.85 ± 0.16 h ($n = 5$, $\chi^2 = 14.50$) and the mortalities of pest adults exposed to the CA for 3 h, 6 h and 9 h were 7.5%, 8.6% and 10.8%, respectively (Table 1).

3.2 Effect of CA treatment on CarE

Exposure time significantly affected the CarE specific activity ($F_{3,8} = 516.51$, $P < 0.01$) and linear regression indicates that enzyme activity was positively correlated with the CA treatment time ($P = 0.004$). The specific activities of CarE were 0.324, 0.439, 0.502 and 0.612 mmol/mg · min at 3 h, 6 h and 9 h after CA treatment, respectively, which were increased by 35.41%, 55.02% and 88.98%, respectively, compared with the control (0 h exposure period, 0.324 mmol/mg · min (Table

2).

CA treatment significantly increased V_{max} for CarE by 26.13%, 31.77% and 57.12% after exposure to CA for 3 h, 6 h and 9 h, respectively, indicating that the catalytic activity was higher under CA treatment ($P < 0.01$). However, the CA treatment had no effect on K_m ($P = 0.78$), suggesting that substrate affinity was unaffected (Table 3).

3.3 Effect of CA treatment on ACP activity

ACP extracts from both CA treated and control groups showed very good linear relationships between extract concentration and enzyme activity. The slope of the regression lines represented the specific activity, expressed as mmol/mg · min. CA treatment increased ACP specific activity from 1.949 without CA to 2.622 mmol/mg · min, 3.361 mmol/mg · min and 4.412 mmol/mg · min at 3 h, 6 h and 9 h after exposure to CA, corresponding to activity increases of 34.53%, 72.45% and 126.37%, respectively (Table 4).

3.4 Effect of CA treatment on GSTs activity

GSTs activity was significantly affected by CA treatment with different exposure time ($F_{3,8} = 652.9$, $P = 0.015$). The activities were increased by 5.40%, 8.40% and 17.59%, respectively, after exposure to CA for 3 h, 6 h and 9 h. Linear regression analysis indicates a significant relationship ($P = 0.001$) between CA treatment time and GSTs activity (Table 5). The effect of CA on GSTs was much milder than on CarE and only was present with longer exposure time (9 h).

Table 1　Bioassay results for the probability of death of pests exposed to the CA

Probability of death	Exposure period (h)	95% Confidence limits	
		Lower	Upper
0.01	3.57 ± 0.07	1.63	5.24
0.10	6.11 ± 0.14	3.72	7.91
0.50	11.85 ± 0.16	9.61	14.03
0.90	22.93 ± 0.22	18.67	33.05
0.99	39.03 ± 0.19	28.56	74.72

Data in the table are means ± *SE* for exposure period.

Table 2　Effect of CA exposure on CarE activity of *Araecerus fasciculatus*

Treatment time (h)	Linear equation	Specific activity (mmol/mg · min)	Activity induction (%)
0	$y = 0.3239x + 0.0032$ ($R^2 = 0.9928$)	0.3239 ± 0.0072 a	100
3	$y = 0.4386x + 0.0017$ ($R^2 = 0.9914$)	0.4386 ± 0.0016 b	135.41
6	$y = 0.5021x + 0.0038$ ($R^2 = 0.9939$)	0.5021 ± 0.0024 c	155.02
9	$y = 0.6121x + 0.0032$ ($R^2 = 0.9999$)	0.6121 ± 0.0046 d	188.98

Data in the table are means ± *SE* and those followed by matching letters differ significantly at $P < 0.01$, $F = 516.513$ (ANOVA, Duncan's multiple range test).

Table 3　Effects of CA on kinetic parameters of CarE from *Araecerus fasciculatus*

Treatment time (h)	V_{max} (mmol/mg · min)	K_m (mmol/L)
0 (CK)	1.917 ± 0.012 a	0.144 ± 0.009a
3	2.418 ± 0.014 b	0.146 ± 0.010a
6	2.526 ± 0.015 c	0.141 ± 0.009a
9	3.012 ± 0.011 d	0.148 ± 0.013 a

Data in the table are means ± *SE* and those followed by matching letters differ significantly at $P < 0.01$, $F = 218.461$ (ANOVA, Duncan's multiple range test).

Table 4　Effect of CA on ACP activity of *Araecerus fasciculatus*

Treatment time (h)	Linear equation	Specific activity (mmol/mg · min)	Activity induction (%)
0	$y = 1.949x - 0.001$ ($R^2 = 0.998$)	1.949 ± 0.024 a	100
3	$y = 2.622x - 0.011$ ($R^2 = 0.968$)	2.622 ± 0.352 b	134.53
6	$y = 3.361x - 0.001$ ($R^2 = 0.993$)	3.361 ± 0.084 c	172.45
9	$y = 4.142x - 0.002$ ($R^2 = 0.984$)	4.142 ± 0.251 d	226.37

Data in the table are means ± *SE* and those followed by matching letters differ significantly at $P < 0.01$, $F = 366.24$, $df = 3$ (ANOVA, Duncan's multiple range test).

Table 5 Effect of CA on GSTs activity of *Araecerus fasciculatus*

Treatment time (h)	Enzyme activity (mmol/mg·min)	Activity increase (%)
0	0.3702 ± 0.0328 a	100.00
3	0.3902 ± 0.0411 a	105.40
6	0.4013 ± 0.0198 a	108.40
9	0.4353 ± 0.0618 ab	117.59

Data in the table are means ± *SE* and those followed by matching letters differ significantly at $P < 0.01$, $F = 652.9$ (ANOVA, Duncan's multiple range test).

4 DISCUSSION

CA treatments have been used to control a wide range of quarantine and storage pests (Calderon and Navarro, 1979, 1980; Gunasekaran and Rajendran, 2005). CA treatment involves manipulation of CO_2, O_2, and N_2 concentrations in the atmosphere surrounding the stored products and pests. Carbon dioxide is an important factor affecting the efficacy of CA treatments with respect to pest mortality (Bond and Buckl, 1979; Calderon and Navarro, 1979, 1980; Cheng *et al.*, 2001). CA stress led to decrease in feeding activity and delayed development of cowpea bruchids (Margam, 2009). Feeding activity gradually decreases as the oxygen level in the container falls, with a concomitant increase in the level of carbon dioxide, when cowpea bruchid was exposed to different combinations of oxygen and carbon dioxide concentrations (Margam, 2009). The reduction in metabolism lessens the pressure on organisms to initiate anaerobic metabolism, which would require very high rates of anaerobic glycolysis and thus lead to rapid exhaustion of carbohydrate reserves while toxic end products accumulate (Hochachka, 1986; Ofuya and Reichmuth, 1998). Some evidence for this physiological pathway was reported in experiments with *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae).

The current study characterized activity changes of 3 detoxification enzyme systems in *A. fasciculatus* under the influence of a sublethal CA treatment with enriched CO_2. The treatments increased the activities of all the three tested enzyme systems. Specifically, CA treatment of enriched CO_2 increased the specific activity of CarE by 31.75% – 50.82% with 3 h – 9 h exposure. These results suggest that the CA treatment with enriched CO_2 increased CarE specific activity towards α-NA, and the effect was stronger as the exposure time increased. Esterase has been shown to be a detoxification enzyme and its enhancement could result in resistance to organophosphate (OP), carbamate and pyrethroid insecticides in a variety of insects or mites (Li *et al.*, 2003). Increased CarE activity in insects resistant to organophosphorus insecticides has been well documented (Wang *et al.*, 1999, 2000; Li *et al.*, 2003). In the Australian sheep blowfly, *Lucilia cuprina* (Newcomb *et al.*, 1997), the carboxylesterase isozyme, E3 (E. C. 3. 1. 1) was closely related to OPs (such as paraoxon and chlorfenvinphos) resistance. It was once reported that the enhanced activity of the detoxifying enzyme carboxylesterase was one of the major biochemical mechanisms responsible for the development of malathion resistance in Dipstera. Resistance to organophosphorous and carbamate insecticides in the field and laboratory generated by point mutation in the Rop-1 gene, which encodes a carboxylesterase, E3, was also observed in the same species (Cheng *et al.*, 2001). It has been demonstrated that there is some interactive relationship between insect CA resistance and chemical pesticide resistance (Wang *et al.*, 1999, 2000). The current research represented our first attempt to understand the enzymological function responses of *A. fasciculatus* to controlled atmosphere.

The CA treatment increased ACP activity of the pest to a certain extent. As a lysosomal enzyme, ACP may have a role in autophagy and cell turnover as well as defence. Some exogenous chemicals, such as high concentrations of limonoids, can affect the activity and other biochemical characteristics of ACP (Nathan, 2006). ACP activity also increased in the haemolymph after inoculation with the entomopathogenic fungus *M. anisopliae* var. *acridum* (Xia *et al.*, 2000). Inhibition of phosphatase activity in the digestive and excretory organs may be responsible for massive excretion of phosphorylated derivatives of nucleoside analogues after their oral administration to insects (Nëmec and Sláma, 1989). However, after studying the relationship between ACP or ALP activity and resistance in *L. bostrychophila*, Wang *et al.* (2000) reported that ACP or ALP was not related to CA resistance of the species.

GSTs activity was also increased by the CA treatment but required longer treatment time (9 h). The results are similar to typical reports in which upregulation of CarE and ACP occurs normally in animals exposed to hypoxia. Upregulation of GSTs activity, however, does not occur normally under hypoxia. These enzymes catalyse conjugation of reduced glutathione via a sulfhydryl group to

electrophilic centres on a wide variety of substrates. This activity not only detoxifies xenobiotics, but it is most important in detoxifying endogenous reactive oxygen (ROX) species. GSTs generally protect organisms against oxidative toxicity. Thus, more detail information is needed to provide an explanation as to why upregulation of GSTs occurs under hypoxia when it does not occur in other animal systems studied, even if only for longer exposure periods (9 h or more). Perhaps GSTs' other function to bind and transport proteins is responsible for this behavior or it is related to the particular class of GSTs present in the insects studied. More research should be carried out to test the hypotheses regarding the molecular and cellular signalling mechanisms underlying resistance or tolerance to hypoxia.

A high CO_2 condition also increased the V_{max} (catalytic activity) of CarE, but had little effect on its affinity to substrates (K_m). Pest resistance to CA was related to a change in the characteristics of esterase (Wang *et al.*, 2000). The lethiferous efficiency of CA to pests may be related to changes in the activity and kinetic parameters of CarE. These results indicated that *A. fasciculatus* had the ability to adjust its detoxification enzyme systems under the stress of high CO_2/low O_2.

Compared with a number of studies devoted to the mortality of insects in response to various CA treatments, studies on mode of action of CA and insect coping mechanisms are relatively scarce (Mitcham *et al.*, 2006). However, some studies have indicated that insects can develop tolerance or resistance to CA treatment by detoxification (Bond and Buckland, 1979; Navarro *et al.*, 1985; Wang *et al.*, 1999, 2000). For example, ACP activities were higher in a CA resistant strain of *Liposcelis bostrychophila* than that in a susceptible strain (Wang, 1997). The lethal efficacy of CA towards pests appears to be closely related to the change in CarE activity, as an increased CarE activity was suggested as playing an important role in CO_2 resistance of *L. bostrychophila* (Wang *et al.*, 1999, 2000). An increased CarE activity under CO_2 treatment was also associated with increased CO_2 tolerance in *Stegobium paniceum* (L.) (Li *et al.*, 2009b). Tolerance to controlled atmospheres has been induced in granary weevils and selected for in laboratory colonies of stored product beetles (Mitcham *et al.*, 2006). The laboratory selected strains of *T. castaneum* tolerant to either hypercarbia or anoxia, a physiological adaptation via general lowering of metabolic intensity or prolonged development periods. The mechanisms of resistance were the reduced energy used and ability to maintain water balance to survive in elevated CO_2 atmospheres and the ability to maintain aerobic metabolism in reduced O_2 atmospheres (Donahaye, 1992). Resistance to reduced O_2 was less clear; it was potentially due to the ability to maintain aerobic metabolism, since survival was not dependent on triacylglycerol levels (Donahaye and Navarro, 2000). The current work suggests that exposure to CA increases CarE activity in *A. fasciculatus*. These results differ from those using conventional insecticides, which usually lead to a lower CarE activity in susceptible insects. Additionally, increased V_{max} of CarE under an enriched CO_2 atmosphere suggests that CarE could hydrolyse toxic products resulting from the CA.

The measurements of detoxification enzyme activity in insects can be influenced by many biotic and abiotic factors. In addition to individual variations, biotic factors include life stages, body parts and hosts. Abiotic factors may include substrate selection, environmental conditions and experimental procedures. Nevertheless, the trend that CA treatment increases detoxification enzyme activity in insects is apparent. Under high CO_2/low O_2 conditions, insects may cope with the stress by increasing the activities of detoxification enzymes.

The current research focused on a new example involving activity changes to detoxification enzymes in *A. fasciculatus*. We can conclude that under the stress of the CA, the catalytic activities of some detoxification enzymes towards substrate were improved, which potentially underlies ability for pests to adjust metabolic activities to extreme conditions. The results of the current study contribute to the pool of knowledge regarding CA resistance mechanisms and provide a fundamental basis for combating CA resistance in insect pests. The present work also provided fundamental information on the some detoxification enzymes and their induction by a CA in stored product pests. This is expected to be useful in understanding the mechanisms underlying the tolerance of CO_2-enriched atmospheres.

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气调胁迫对咖啡豆象三种解毒酶活性与动力学参数的影响

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摘要：为了分析气调处理对实验昆虫解毒酶性质的影响，探讨酶活性变化与气调抗性形成的潜在关系，本研究用高浓度二氧化碳胁迫处理咖啡豆象 *Araecerus fasciculatus*（De Geer），研究其羧酸酯酶（carboxylesterase，CarE）、酸性磷酸酯酶（acid phosphatase，ACP）和谷胱甘肽转移酶（glutathione S-transferases，GSTs）的生物化学与毒理学性质。结果表明：高浓度二氧化碳气调胁迫处理咖啡豆象 3 h，6 h 和 9 h，其 CarE 酶活力分别升高 35.41%，55.02% 和 88.98%。CarE 酶促动力学参数 V_{max} 分别升高 26.13%，31.77% 和 57.12%，K_m 没有显著改变；相应处理下，ACP 活力分别升高 34.53%，72.45% 和 126.37%；GSTs 活力分别升高 5.40%，8.40% 和 17.59%。可见，二氧化碳气调胁迫下，实验昆虫可以通过调节部分解毒酶的酶活力或酶促动力学参数来应对不利环境；酶与底物间的亲和力增强可能是昆虫在气调胁迫下保护机体免受伤害的一种代谢反馈信息。本研究可为昆虫气调杀虫及其抗（耐）气性形成机制提供一些基础信息。

关键词：储藏物害虫；咖啡豆象；气调；气调抗性；解毒酶；酶活性；酶动力学

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